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Regular Article

Biochemical Characterization of an ecotype cichlid from Epe Lagoon 'Wesafu' and *Oreochromis niloticus* using Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Hammed, A.M.[1], Fashina-Bombata, H.A.[1] and Omitogun, G.O.[2]

[1]Department of Fisheries, Faculty of Science, Lagos State University, PMB 1087, Apapa, Lagos-Nigeria
[2]Department of Animal Science, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
Correspondence author: bombatta2002@gmail.com

The abundance of a yet to be identified cichlid in Epe Lagoon commonly referred to as Wesafu and the large size/weight it attains in the wild elicited interest in the fish for domestication, culture and possible species identification and naming. Characterization of Wesafu and *O. niloticus* biochemically using SDS-PAGE was carried out to analyze protein mixtures in the two species qualitatively and to determine their relative protein molecular mass. After centrifugation for 15min at 1000 rpm, blood sera from the two species were electrophoretically run simultaneously on 5.5% SDS polyacrylamide gel following the procedure of [Avtalion et al., (1976); Betiku and Omitogun, (2006)]. After electrophoretic separation, the gel was stained, destained, sealed in polythene bags and refrigerated. Gels produced were scored visually with presence (1) or absence (0) of band classifications. Frequencies were determined by direct counting while genetic interpretation was based on frequency and relative banding intensity. Distinct bands (genetic markers) were observed between the two species. While Wesafu had between 10 and 11 bands, 9 bands was recorded for *O. niloticus*. The 11th band in Wesafu was identified as a post albumin which is absent in *O. niloticus*. In *O. niloticus*, bands 1, 3 and 7 were present, but bands 2, 5, 6 and 8 were absent in few. Some individuals of Wesafu were found to possess weaker bands 3 and 7. The band frequency between samples studied indicated serum protein polymorphism, which indicated genetic diversity between 'Wesafu' and *O. niloticus*.

Key words: Wesafu, *O. niloticus*, electrophoresis.

The bulk of systematic information in fish, including the tilapias, is based on measurements and counts of comparable body parts and characters (Thys van den Audenaerde 1970; Trewavas 1983; Teugels and Thys van den Audenaerde 1992). The theory, practice and results of these are known as morphological systematics. It is, however, accepted that characters upon which morphological systematics are based may be influenced by the environment.

To reduce the overall influence of environment on systematic information, biologists have borrowed approaches from protein chemists. This has mainly involved the analysis of deoxyribonucleic acid (DNA)

or of the primary product (protein) from which codes is expressed so as to provide information on biochemical character, status of individuals and populations within and among taxonomic units.

Nuttal (1901) suggested some advantages in the use of biochemical characters in support or to define morphological systematics having been long recognized. These approaches comprised immunological and various electrophoretic techniques, peptide analysis and amino acid sequencing.

New species are genetic resources, within the communities and ecosystems that house them. Many of their useful or potential attributes are coded in genes, conservation and identification are a necessary first step in the study new species through the branch of science known as taxonomy or systematic. There are still many aquatic species to be accurately identified and their populations evaluated for aquaculture potential. Amongst the fin fishes, the tilapias are generating high interest for aquaculture around the world.

Materials and Methods

Blood sample collection and serum preparation

Eleven live specimens of Wesafu with a weight range of 570 to 900g and six live specimens of *O. niloticus* (180-450g) were transported to Lagos State University Department of Fisheries laboratory where blood samples for assay were collected by means of heparin-lined syringe (2-5ml). A 0.9ml of blood drawn from the caudal peduncle of the fish and 0.6ml (3/2) of physiological salt solution was added directly into serological tube furnished with a long wooden spill-around.

Clot was allowed to form in about 1hr at room temperature. The clot was removed and centrifugation was carried out at 1000rpm for 15min. The serum protein was carefully transferred into a clean 2ml Eppendorf micro tube and stored at -35^{o}C (Avtalion, 1984) for further analyses at the Biotechnology Laboratory of the Department of Animal Science, Obafemi Awolowo University, Ile-Ife. The residual erythrocytes were discarded since haemolytic sera are usually not well separated electrophoretically (Avtalion, 1984; Betiku and Omitogun, 2006).

Centrifugation

The supernatant from centrifugation which contained serum protein was frozen for gel electrophoresis.

Sample dilution

A small quantity (0.1ml) of 1:2 saline-diluted serums was further diluted (1:3) in 40% sucrose solution (to a final1:6). A small drop (2µl) of bromophenol blue was added as an indicator for the electrophoretic mobility.

Electrophoretic procedure

10 microliter (50µg protein) sucrose bromophenol solution was introduced into the gel hole, using a micro syringe; since this solution is known to rapidly denatured within 24 – 28 hours, the procedure was carried out immediately.

Electrophoretic field

During the first 30 minutes, electric voltage was kept constant at 180V allowing a direct current of 2-3mA/cm gel after which the current was decreased to 150V for 45 minutes to allow gel separation (Avtalion *et al*., 1976).

Staining and destaining of gels

After electrophoretic separation, the gel was gently removed from the apparatus and put into a staining solution made from 40ml of ethanol, 10ml of glacial acetic acid and 0.1g of freshly prepared powdered Coomasie blue dissolved in 100ml of distilled water for 18 hours. The gel was then destained in destaining solution containing 60ml distilled water, 40ml ethanol and 10ml glacial acetic

acid left for several days until the background was completely clear.

Storage of gel

The gels were sealed into polythene bags containing small quantity of distilled water and refrigerated.

Scoring of electrophoretic bands

The locus and allele nomenclature was adopted (Allendorf and Utter, 1979). Gels were scored visually. Presence (1) or absence (0) of protein bands classification was used. Frequencies were determined by direct counting and the genetic interpretations based on frequency and relative banding intensity (Betiku and Omitogun, 2006).

Statistical Analysis

Data generated were analyzed using PAST (PAleontological STatistics) software package (version 2.0) to generate a dendrogram for the samples (Hammer *et al*., 2001).

Results

The representative electrophoretic profile of the serum proteins of 'Wesafu' and *Oreochromis niloticus* are shown in figure 1 and table i. Albumin was the fastest migrating band and had the highest staining intensity, while transferrins showed the intermediate bands and the slowest migrating bands which represented the biggest protein molecule were globulins found close to the well comb (origin). The relative mobility (RM) of each protein band (tables 2 and 3) was calculated using the albumin band as a reference point (RM of 1). Cluster analysis of the paired group coefficient of genetic similarities between the two species is presented in figure 2.

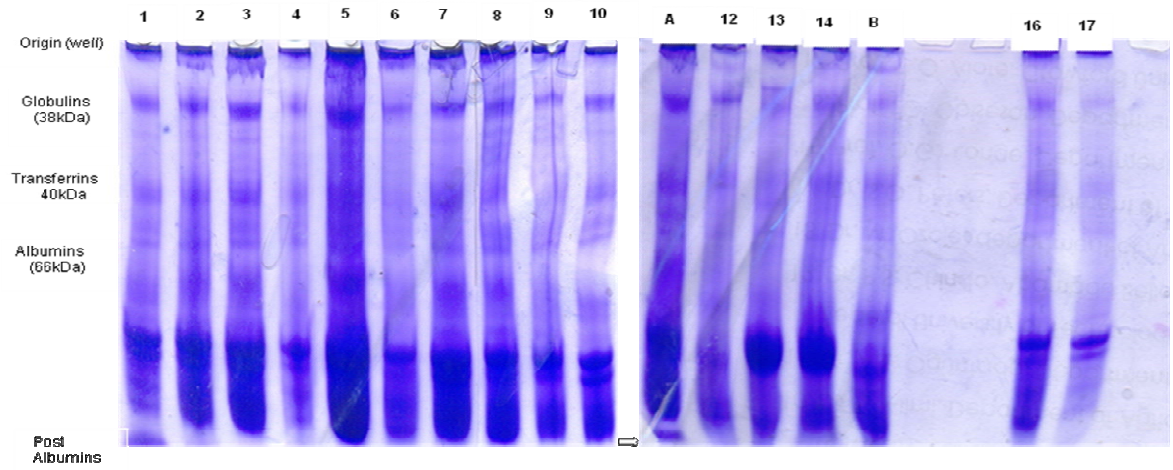

Figure 1: 5.5% SDS-polyacrylamide gel Coomassie-stained electrophoregram of sera Collected from Wesafu and *O. niloticus*. Molecular weight markers as indicated at the extreme left of the picture are expressed in kilodaltons (kDa). Accessions used for the two species are: Wesafu (1-10 with A as standard) and *O. niloticus* (12, 13, 14, 16, 17 with B as standard) respectively.

Discussion

Tilapia is a common name for more than 70 species of perch-like fishes in Cichlid family, native to freshwater tropical Africa (Trewavas, 1983; Stiassny, 1991). These fishes have been introduced into nearly every tropical and sub-tropical country of the world to support the development of freshwater aquaculture to an estimated world production exceeding 659,000 t/yr

(FAO, 2002). The contribution of Nigeria's cichlid aquaculture to world total output is nil (Fashina-Bombata *et al.*, 2006). Tilapia aquaculture in the country is not attractive for reasons which include stunting, low market value, and the lack of developed commercially viable species. Research into the development of a commercial strain has not been conducted and this, in turn, is responsible for the lull in the Cichlid aquaculture industry in the country (Fashina-Bombata *et al.*, 2010; Hammed *et al.*, 2010). Identification of individual species of fish is therefore, very important in aquaculture production as this help in maintaining the purity of discrete stock and introgression of the cultured fishes into the wild (Betiku and Omitogun, 2006).

Table I: Observed bands in the blood sera samples of Wesafu and *O. niloticus*

Fish Sample	Number of Bands										
	a	b	c	d	e	f	G	h	i	j	k
	Glob	Glob	Tf	Tf	Tf	Al	Al	Al	Al	Al	Pst. Al
1	1	1	0	1	0	1	1	1	1	1	0
2	1	1	0	0	0	1	0	1	1	1	0
3	1	1	1	1	1	1	1	1	1	1	0
4	1	1	0	0	0	0	0	0	0	1	0
5	1	1	0	0	0	0	0	0	0	1	0
6	1	1	0	0	0	0	0	0	0	1	0
7	1	1	0	1	1	1	0	1	0	1	0
8	1	1	0	1	1	0	0	1	0	1	0
9	1	1	0	1	0	0	0	0	0	1	0
10	1	1	0	1	0	1	1	1	0	1	0
11	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	0	0	1	0	1	0	0
13	1	0	1	0	0	0	1	0	1	0	0
14	1	0	1	1	1	0	1	0	1	0	0
15	1	1	1	1	1	1	1	1	1	0	0
16	1	0	1	1	0	1	1	0	1	0	0
17	1	0	1	1	1	0	1	1	0	0	0

Legend: A-K (number of bands); Glob (Globulin); Tf (Transferin); Al (Albumin); Pst. Al (Post Albumin)

Table I indicates the position and number of bands in the sera of samples from Wesafu and *O. niloticus*. It shows that all specimens of Wesafu (1-10 and A) analyzed have the first and second globulin (1st and 2nd bands) as well as the 10th band (Albumin). Transferin (3rd to 5th bands) varies with individual specimen just as 6th to 9th bands (Albumin) also varies. None except specimen 11 has post albumin band which is very rare in most species (Betiku, *et al;* 2006). It shows that all specimens of *O. niloticus* (12, 13, 14. B, 16, 17) analyzed have the first and seventh bands (globulins and albumins). Nine (9) bands were identified in *O. niloticus* with bands 2, 6 and 8 either absent or weaker in few individuals. There is neither band 10 nor 11 (post albumin) as found in Wesafu, while only specimen 15 has all the 9 bands intact.

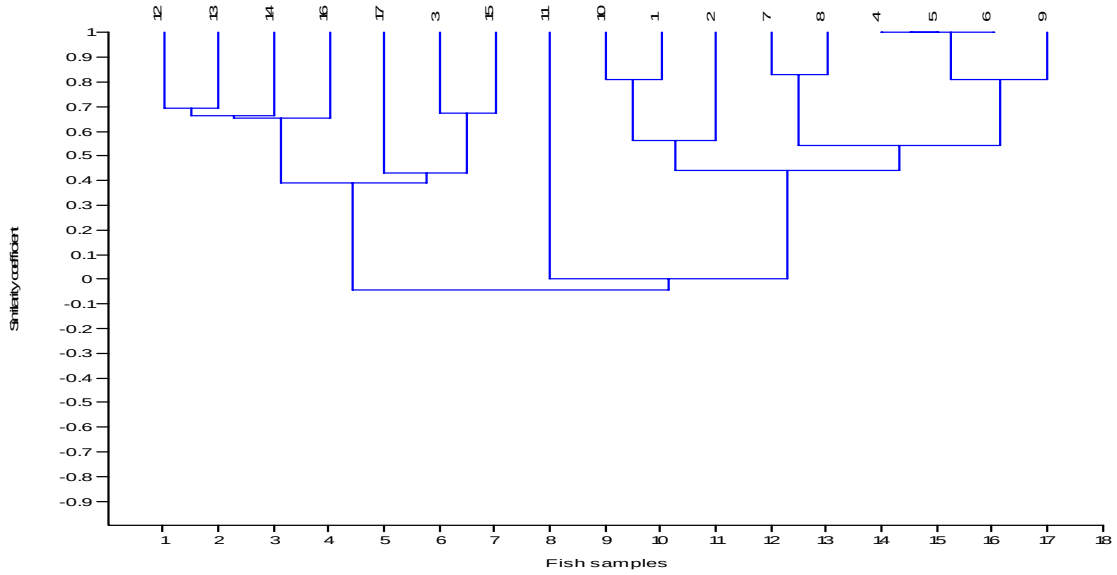

Fig. 2: Dendrogram produced by PAST cluster analysis, showing the paired group correlation coefficient of genetic similarities in the samples of Wesafu and *O. niloticus*.

Table II: Frequencies (Calculated as the percentage of fish samples that showed the band) and relative mobilities (RM) of serum protein in Wesafu

Bands	a	b	c	d	e	f	G	H	i	j	k
Proteins	Glob	Glob	Tf	Tf	Tf	Al	Al	Al	Al	Al	Pst. Al
Frequency	1.00	1.00	0.18	0.64	0.36	0.55	0.36	0.64	0.36	1.00	0.09
RM (A)	0.03	0.15	0.36	0.49	0.53	0.59	0.74	0.77	0.86	0.93	1.00

Legend: A-K (number of bands); Glob (Globulin); Tf (Transferin); Al (Albumin); Pst. Al (Post Albumin)

Table II shows the relative mobility of the serum protein band for Wesafu. Globulin present in all specimens, the number of transferrin and albumin varies, while only specimen 11 was observed to possess 11 bands (a post albumin band), which reflects the heterozygosity of wild samples i.e. supporting the theory of the wide genetic diversity in wild populations.

Table III: Frequencies (calculated as the percentage of fish samples that showed the bands) and relative mobility of serum protein in *O. niloticus*

Bands	A	b	C	D	e	F	G	h	I
Proteins	Gl	Gl	Tf	Tf	Tf	Al	Al	Al	Al
Frequency	1.00	0.33	1.00	0.83	0.50	0.33	1.00	0.33	0.83
RM (B)	0.06	0.15	0.36	0.49	0.76	0.85	0.88	0.91	1.00

Legend: A-K (number of bands); Glob (Globulin); Tf (Transferin); Al (Albumin); Pst. Al (Post Albumin)

Table III shows the relative mobility of the serum protein band for *O. niloticus*. 1st globulin was present in all specimens of *O. niloticus*, while only two specimens were found to possess the 2nd globulin, but numbers of transferrin and albumin varies. Nine (9) bands were however observed in species of *O. niloticus*.

The electrophoretic study was an attempt to characterize Wesafu and compare it with a very similar type, *O. niloticus* using SDS polyacrylamide gel electrophoresis assay developed and found to be useful in the identification of cichlid species and hybrids (Avtalion *et al.* 1975; Herzberg, 1978; Cruz *et al*., 1982; MacAndrew and Majumdar, 1983; Wu and Wu, 1983; Basiao and Taniguchi, 1984). The study using transferrin marker was based on the presence of bands 10 and 11 (table 1) in Wesafu and their total absence in *O. niloticus*. However, in *O. niloticus*, bands 1, 3 and 7 are always present, whereas bands 2, 5, 6 and 8 are either absent or weaker in few individuals. This finding is supported by Galman *et al.,* (1988) who reported absence of band 6 in few individuals of *O. niloticus*, but weaker band 8. Some specimen of Wesafu were however, found to have weaker bands 3 and 7. The frequency of occurrence and relative mobility of the serum protein bands for both species are quite different (Tables II and III). While the frequency of occurrence in Wesafu ranges between 0.09 (post albumin) to 1.00 (1st and 2nd globulin, that of *O. niloticus* ranges between 0.33 (2nd globulin, 4th transferin and 3rd albumin) to 1.00 (1st globulin). The relative mobility indicates that of Wesafu to range between 0.03 (1st globulin) to 1.00 (post albumin) and 0.06 (1st globulin) to 1.00 (4th albumin) in O. niloticus. The post albumin band in Wesafu is an indication of different genetic composition. The scored bands were further analyzed using PAleontological STatistics (PAST) software package (version 2.0) as described by Hammer *et al.,* (2001) to confirm earlier findings and to determine the paired group coefficient of genetic similarities in the samples and the standard genetic distances between the two species. The result dendrogram (figure 2) reveals a high level of genetic diversity between the fish sampled and at the same time support the theory of heterozygosity.

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